

Data Evaluation Report on the Acute Toxicity of Limonene to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number { }

EPA MRID Number 49044002

Data Requirement: PMRA DATA CODE { }
EPA DP Barcode 409159
OECD Data Point { }
EPA MRID 49044002
EPA Guideline 850.4500

Test material: Avenger Weed Killer Concentrate **Purity:** 70% a.i.
Common name: Limonene
Chemical name: IUPAC: Not reported
 CAS name: Not reported
 CAS No.: Not reported
 Synonyms:

Primary Reviewer: Kindra Bozicevich
Environmental Scientist, CDM Smith

Signature: *Kindra Bozicevich*
Date: 02/12/14

Secondary Reviewer: Teri S. Myers
Environmental Scientist, CDM Smith

Signature: *Teri S. Myers*
Date: 03/05/14

Primary Reviewer: Katherine Stebbins
EPA

Date: 02/22/18 *Katherine Stebbins*

EPA PC Code 079701

Date Evaluation Completed: 02/22/18

CITATION: Mikulas, J. 2013. Avenger Weed Killer Concentrate Algal Toxicity Tier II Test with *Pseudokirchneriella subcapitata*. Study performed by Stillmeadow, Inc., Sugar Land, TX. Study ID 16462-12. Study sponsored by Cutting Edge Formulation, Buford, GA and submitted by MacIntosh & Associates, Inc. Study initiated 02 October 2012 and completed 11 January 2013.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of freshwater green algae, *Pseudokirchneriella subcapitata* (strain not reported) were exposed to Avenger Weed Killer Concentrate (70% ai d-limonene) at nominal concentrations of 0 (negative control), 5, 10, 20, 40, and 80 mg ai/L under static conditions. Mean measured concentrations were not reported as the test concentrations were not analytically-determined. The NOAEC value based on all parameters was 20 mg ai/L, in terms of nominal concentrations. The EC₅₀/IC₅₀ values based on yield, growth rate, and area under the curve were 37.31, 178.7, and 36.75 mg ai/L, respectively, in terms of nominal concentrations. The % growth inhibition of cell density in the treated algal culture as compared to the control ranged from 5 to 79%.

After 96 hours, the cells in the 40 mg/L test group appeared small and cells in the 80 mg/L test group appeared small and misshapen. There was an increase in pH during the study.

The test concentrations were not analytically determined in this study and, as a result, all toxicity values are based upon nominal concentrations and the stability is not verified. Due to the volatility of the test compound, there is uncertainty with use of the nominal concentrations. Additionally, there is a lack of information describing the materials and methods used and this study only had three replicates. Submission of data to support the stability of the formulation under test conditions and further description of any other methods used that could have potentially reduced the volatilization of a.i. over the test (e.g., closed flask), would reduce the uncertainty related to exposure. This study is classified as **SUPPLEMENTAL** and provides data that are useful for characterization purposes.

Results Synopsis

Test Organism: Freshwater green algae, *Pseudokirchneriella subcapitata* (strain not reported)

Test Type (Flow-through, Static, Static Renewal): Static

Yield

EC₀₅: 8.631 mg ai/L 95% C.I.: N/A to 18.54 mg ai/L
EC₅₀: 37.31 mg ai/L 95% C.I.: 25.04 to 55.59 mg ai/L
NOAEC: 20 mg ai/L

Probit Slope: N/A

Growth rate

EC₀₅: 1.917 mg ai/L 95% C.I.: N/A to 22.71 mg ai/L
EC₅₀: 178.7 mg ai/L 95% C.I.: 8.885 to 3593 mg ai/L
NOAEC: 20 mg ai/L

Probit Slope: N/A

Area under the curve (AUC)

EC₀₅: 11.28 mg ai/L 95% C.I.: N/A to 20.25 mg ai/L
EC₅₀: 36.75 mg ai/L 95% C.I.: 26.48 to 50.99 mg ai/L
NOAEC: 20 mg ai/L
Probit Slope: N/A

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was designed to comply with the procedures of the U.S. EPA OCSPP 850.4500 guideline. The following deviations from the U.S. EPA OCSPP 850.4550 Guideline (2012) are noted:

1. The stability of the test material under test conditions was not assessed. As a result, mean measured test concentrations were not determined.
2. The original source and strain of the test organism were not reported. It was noted that the stock cultures were maintained in-house, but the amount of time that the specific culture used in the test had been cultured in-house was not reported. Newly obtained cultures should be maintained in-house for at least 6 weeks prior to use in testing.
3. The health/condition of the inoculum culture was not described. The inoculum should be from a logarithmically growing stock culture.
4. The type of material used for the test containers and the fill volume for each test vessel was not reported. The test volume should not exceed 50% of the flask volume.
5. The source/type, pH, water pretreatment (if any), total organic carbon, particulate matter and chlorine concentrations of the dilution water were not reported.
6. The chelator used and the carbon source for the AAP medium was not reported.
7. The method used to prepare the test solutions for the definitive test was not specified. The test material should be added to nutrient medium by either direct addition or by addition of a stock solution.
8. Only 3 replicates were used per treatment level (and 6 for the control). A minimum of 4 replicates per level is recommended.
9. The test exposure concentrations were not analytically verified.
10. The quality of the light source was not reported. Cool-white fluorescent light is recommended.
11. The study author did not provide measurement techniques for growth rate or area under the curve.

These deviations affect the validity of the study.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study was performed in accordance with the GLP standards of U.S. EPA (40 CFR Part 160) with the following exceptions: Section 160.31(d) and 160.105(a)(b)(e) Characterization and stability information was not provided to the testing facility. Section 160.113(a) Mixture analysis was not performed.

A. MATERIALS:

1. Test material Avenger Weed Killer Concentrate

Description: Slight yellow liquid

Lot No./Batch No. : Lot# B186

Purity: 70% a.i.

Stability of compound under test conditions: Not assessed.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test

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compound)

Storage conditions of test chemicals: Room temperature.

Physicochemical properties of Limonene.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Freshwater green algae, *Pseudokirchneriella subcapitata*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricornutum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not reported
Source: In-house cultures (original source not reported)
Age of inoculum: 3-7 ± 1 days prior to dosing
Method of cultivation: Cultured in AAP medium at 24 ± 2 °C under continuous light (4300 ± 645 lux) and continuously shaken at 100 cycles/minute.

B. STUDY DESIGN:

1. Experimental Conditions

- a. Range-finding study: A preliminary range-finding test was performed with test concentrations of 1, 10, 100, 500, and 1000 mg/L. The algae were introduced at an initial cell density of 25,000 cells/mL, and at 96 hours the terminal density was measured. Based on the results of the preliminary test, the concentrations selected for the definitive study were 5, 10, 20, 40, and 80 mg/L.
- b. Definitive Study

Table 1: Experimental Parameters

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Parameter	Details	Remarks
		<i>Criteria</i>
Acclimation period:	Continuously cultured in-house	
Culturing media and conditions: (same as test or not)	Same as test	
Health: (any mortality observed)	None reported	<p><i>EPA recommends two week acclimation period.</i></p> <p><i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i></p>
<u>Test system</u> Static/static renewal	Static	
Renewal rate for static renewal	N/A	<p><i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i></p>
Incubation facility	Temperature-controlled cabinet held within an environmental chamber	
Duration of the test	96 hours	<p><i>EPA requires: 96-120 hours</i></p> <p><i>OECD: 72 hours</i></p>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Not reported 250 mL Not reported	<p>Erlenmeyer flasks</p> <p><i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i></p>
<u>Details of AAP medium</u>		

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Parameter	Details	Remarks
		Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	6.0 (at 80 mg/L) to 7.5 (control) 8.2 to 9.4 Not reported Not reported N/A	<i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i> <i>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	A standard medium was used. A detailed composition was not provided.	
<u>Dilution water</u> source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Not reported Not reported N/A Not reported Not reported Not reported None expected at concentrations considered to be toxic None expected at concentrations considered to be toxic Not reported	<i>EPA pH: <u>Skeletonema costatum</u>= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</i> <i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Not specified. In the preliminary study, the test material was added directly to the medium.	
Aeration or agitation	100 cycles/minute by shaker table	

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Parameter	Details	Remarks
		<i>Criteria</i>
Initial cells density	5.0x10 ⁴ cells/mL	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
<u>Number of replicates</u>		
Control:	6	
Solvent control:	N/A	
Treatments:	3	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</p> <p>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</p>
<u>Test concentrations</u>		
Nominal:	0 (negative control), 5, 10, 20, 40, and 80 mg/L	<p>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</p>
Measured:	N/A	<p>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</p>

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Parameter	Details	Remarks
		Criteria
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	N/A	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	24 ± 2°C Continuous 4100 lux (quality not reported)	<p><i>EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)</i></p> <p><i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i></p>
<u>Reference chemical (if used)</u> name: concentrations:	Zinc chloride 10 mg/L	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density Growth rate Area under the growth curve	<p><i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i></p>

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Parameters	Details	Remarks
		Criteria
Measurement technique for cell density and other end points	Cell density was determined using a hemacytometer. The study author did not provide measurement techniques for growth rate or area under the curve.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	Every 24 hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	Cells were visually observed for health.	
Indicate whether there was an exponential growth in the control	Yes, after 96 hours, the mean cell density of the negative control was 351.0×10^4 cells/mL	<i>EPA requires control cell count at termination to be $\geq 2X$ initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours, the mean cell density of the negative control was 351.0×10^4 cells/mL, yielding inhibitions relative to the negative control of 14, 40, 5, 66, and 79% for nominal concentrations of 5, 10, 20, 40, and 80 mg/L, respectively. The NOAEC and EC50 values reported by the study author based on cell density were <5 and 38.747 mg/L, respectively, in terms of nominal concentrations.

The mean 0-96 hour yield of the negative control was 346.0×10^4 cells/mL, yielding inhibitions relative to the negative control of 14, 40, 5, 67, and 80% for nominal concentrations of 5, 10, 20, 40, and 80 mg/L, respectively. The study author did not assess yield values.

The mean 0-96 hour growth rate of the negative control was 0.0442/hour, yielding inhibitions relative to the negative control of 3, 34, 1, 39, and 37% for nominal concentrations of 5, 10, 20, 40, and 80 mg/L, respectively. The NOAEC and EC50 values reported by the study author based on growth rate were <5 and >80 mg/L, respectively, in terms of nominal concentrations.

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The mean 0-96-hour area under the curve (AUC) value of the negative control was 8176, yielding inhibitions relative to the negative control of -16, 41, -2, 64, and 85% for nominal concentrations of 5, 10, 20, 40, and 80 mg/L, respectively. The NOAEC and EC50 values reported by the study author based on AUC were 20 and 37.121 mg/L, respectively, in terms of nominal concentrations.

After 96 hours, the cells in the 40 mg/L test group appeared small and cells in the 80 mg/L test group appeared small and mishappen. There was an increase in pH during the study.

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Table 3: Effect of Avenger Weed Killer Concentrate on algal growth (freshwater green algae, *Pseudokirchneriella subcapitata*)

Treatment Nominal concentrations mg/L	Initial cell density ($\times 10^4$ cells/mL)	Cell density ($\times 10^4$ cells/mL) at			
		48 hours	72 hours	96 hours	
				cell count	% inhibition ^a
Negative control	5	46.5	121.2	351.0	N/A
5	5	54.3	192.3	301.7	14
10	5	27.3	76.7	212.0	40
20	5	34.0	152.0	334.7	5
40	5	35.0	40.0	119.0	66
80	5	5.3	23.0	73.3	79
Reference chemical (zinc chloride)		Cell density was 0 $\times 10^4$ cells/mL after 96 hours.			

^a Calculated by the reviewer relative to the negative control.

Table 4: Effect of Avenger Weed Killer Concentrate on algal growth (freshwater green algae, *Pseudokirchneriella subcapitata*)

Treatment Nominal concentrations mg/L	Initial cell density ($\times 10^4$ cells/mL)	Mean growth rate (hour ⁻¹) ^a		Mean area under the curve (AUC) ^b		Mean yield (based on cell density; $\times 10^4$ cells/mL) ^c	
		0-96 hours	% inhibition ^d	0-96 hours	% inhibition ^d	0-96 hours	% inhibition ^d
Negative control	5	0.0442	N/A	8176	N/A	346.0	N/A
5	5	0.0427	3	9512	-16	296.7	14
10	5	0.0294	34	4836	41	207.0	40
20	5	0.0438	1	8340	-2	329.7	5
40	5	0.0270	39	2920	64	114.0	67
80	5	0.0279	37	1260	85	68.3	80

^a Values reported here are those calculated by the reviewer as the change in the natural logarithm of cell density from 0 to 96 hours, divided by 96 hours. The growth rate values reported by the study author omitted some values that were considered as outliers and were not used in the study author's statistical analysis. The study author reported mean growth rate values of 0.0447, 0.0427, 0.0432, 0.0438, 0.0369, and 0.0279/hour for the negative control and nominal concentrations of 15, 10, 20, 40, and 80 mg/L, respectively.

^b Values reported here are those calculated by the reviewer as the area under the cell density growth curve. The AUC values reported by the study author omitted some values that were considered as outliers and were not used in statistical analysis. The study author reported mean AUC values of 8251.2, 9512, 7044, 8340, 3894, and 1260 for the negative control and nominal concentrations of 15, 10, 20, 40, and 80 mg/L, respectively.

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^c Calculated by the reviewer as 96 hour cell density minus initial cell density.

^d Calculated by the reviewer relative to the negative control.

Table 5: Statistical endpoint values.* (calculated by the study author based on nominal concentrations)

Statistical endpoint	Cell density	Yield	Growth rate	Area under the curve (AUC)
NOAEC (mg/L)	<5	Not calculated	<5	20
LOAEC (mg/L)	5	Not calculated	5	40
IC ₀₅ or EC ₀₅ (mg/L) (95% C.I.)	1.905 (1.324-3.045)	Not calculated	22.440 (19.848-25.323)	6.887 (5.989-8.898)
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	38.747 (36.673-41.410)	Not calculated	>80 (N/A)	37.121 (32.628-41.634)
Reference chemical NOAEC IC ₅₀ /EC ₅₀ (95% C.I.) (mg/L)	Not reported.			

* Do not use this table, if the study was deemed unacceptable.

N/A: Not applicable

B. REPORTED STATISTICS:

The study author statistically analyzed all endpoints using ToxCalc Version 5.0™ statistical software. The EC values were determined using one of the following methods: Probit, Trimmed Spearman-Karber, and Linear Interpolation. All analyses were based on nominal concentrations.

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C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically analyzed the endpoints for yield, growth rate, and AUC using CETIS version 1.8.7.12 statistical software with backend database settings implemented by EFED on 31 May 2013. Area under the curve (AUC) data were confirmed to be normally distributed using Shapiro-Wilk's test, but had unequal variances according to Bartlett's test and was therefore analyzed using Mann-Whitney U Two-Sample test. Yield and growth rate were not distributed normally and had unequal variances and were therefore analyzed using Mann-Whitney U Two-sample test. The ECx values were calculated using Bruce-Versteeg regression. All analyses were based on nominal concentrations.

Yield

EC₀₅: 8.631 mg ai/L 95% C.I.: N/A to 18.54 mg ai/L
EC₅₀: 37.31 mg ai/L 95% C.I.: 25.04 to 55.59 mg ai/L
NOAEC: 20 mg ai/L

Probit Slope: N/A

Growth rate

EC₀₅: 1.917 mg ai/L 95% C.I.: N/A to 22.71 mg ai/L
EC₅₀: 178.7 mg ai/L 95% C.I.: 8.885 to 3593 mg ai/L
NOAEC: 20 mg ai/L

Probit Slope: N/A

Area under the curve (AUC)

EC₀₅: 11.28 mg ai/L 95% C.I.: N/A to 20.25 mg ai/L
EC₅₀: 36.75 mg ai/L 95% C.I.: 26.48 to 50.99 mg ai/L
NOAEC: 20 mg ai/L

Probit Slope: N/A

D. STUDY DEFICIENCIES:

The test concentrations were not analytically-determined in this study and, as a result, all toxicity values are based upon nominal concentrations. This deficiency along with the use of an End use product (70% a.i.), rather than TGAI led to a classification of SUPPLEMENTAL. There were also several other guideline deviations and reporting deficiencies noted in the Materials and Methods section of this DER.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions did not agree with those of the study author for growth rate data; the study author did not assess yield values. The reviewer included in the statistical analysis a replicate in the negative control and a replicate in the 40 mg/L treatment group that the study author considered to be outliers and was not used in statistical analysis. The reviewer's conclusions were in agreement with those of the study author for AUC data. The reviewer's results are presented in the Executive Summary and Conclusions sections of this report.

The laboratory portion of the definitive test was conducted from 19 to 23 October 2012.

The coefficient of variation (CV) based on yield for the negative control was 10.93%, which meets the guideline requirement of yield CV<20%. The CV based on growth rate for the negative control was 2.67%, which meets the guideline requirement of growth rate CV<12%.

F. CONCLUSIONS:

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This study is classified as SUPPLEMENTAL. After 96 hours, the NOAEC value based on all parameters was 20 mg ai/L, in terms of nominal concentrations. The most sensitive endpoint was area under the curve with an EC₅₀/IC₅₀ value of 36.75 mg ai/L.

Yield

EC₀₅: 8.631 mg ai/L 95% C.I.: N/A to 18.54 mg ai/L
EC₅₀: 37.31 mg ai/L 95% C.I.: 25.04 to 55.59 mg ai/L

NOAEC: 20 mg ai/L

Probit Slope: N/A

Growth rate

EC₀₅: 1.917 mg ai/L 95% C.I.: N/A to 22.71 mg ai/L
EC₅₀: 178.7 mg ai/L 95% C.I.: 8.885 to 3593 mg ai/L

NOAEC: 20 mg ai/L

Probit Slope: N/A

Area under the curve (AUC)

EC₀₅: 11.28 mg ai/L 95% C.I.: N/A to 20.25 mg ai/L
EC₅₀: 36.75 mg ai/L 95% C.I.: 26.48 to 50.99 mg ai/L

NOAEC: 20 mg ai/L

Probit Slope: N/A

Endpoint(s) Effected: yield, growth rate, area under the curve

III. REFERENCES: None reported.

CETIS Summary Report

Report Date: 11 Feb-14 12:31 (p 1 of 2)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity**Stillmeadow, Inc.**

Batch ID: 08-7333-8569	Test Type: Algal Cell Growth (96-h)	Analyst:
Start Date: 19 Oct-12	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)	Diluent: Algal Culture Media
Ending Date: 23 Oct-12	Species: Pseudokirchneriella subcapitata	Brine:
Duration: 96h	Source: Lab In-House Culture	Age: 3-7
Sample ID: 18-2731-0145	Code: 49044002	Client: CDM Smith
Sample Date: 19 Oct-12	Material: Limonene	Project: Unknown
Receive Date:	Source: MacIntosh & Associates (MacIntosh)	
Sample Age: NA	Station:	

Batch Note: 079701 49044002 static**Sample Note:** 079701 49044002**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-2946-8890	96h AUC	20	40	28.28	NA		Jonckheere-Terpstra Step-Down Test
07-7830-0042	96h AUC	20	40	28.28	26.5%		Mann-Whitney U Two-Sample Test
18-6441-3558	96h Cell Density	20	40	28.28	NA		Jonckheere-Terpstra Step-Down Test
01-3284-6640	96h Cell Density	20	40	28.28	29.9%		Mann-Whitney U Two-Sample Test
04-1742-7163	96h Growth Rate	20	40	28.28	NA		Jonckheere-Terpstra Step-Down Test
19-0981-3494	96h Growth Rate	20	40	28.28	32.6%		Mann-Whitney U Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
04-7375-0817	96h AUC	IC5	11.28	N/A	20.25		Nonlinear Regression
		IC10	14.64	N/A	24.17		
		IC25	22.64	9.416	34.88		
		IC50	36.75	26.48	50.99		
12-9972-1746	96h Cell Density	IC5	8.631	N/A	18.54		Nonlinear Regression
		IC10	11.93	N/A	22.77		
		IC25	20.47	6.377	35.4		
		IC50	37.31	25.04	55.59		
08-1788-5182	96h Growth Rate	IC5	1.917	N/A	22.71		Nonlinear Regression
		IC10	5.22	N/A	37.16		
		IC25	27.83	6.243	81.62		
		IC50	178.7	8.885	3593		

CETIS Summary Report

Report Date: 11 Feb-14 12:31 (p 2 of 2)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity**Stillmeadow, Inc.****96h AUC Summary**

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	6	8176	7402	8950	7416	9324	301.3	738	9.03%	0.0%
5		3	9512	7854	11170	8772	10070	385.3	667.3	7.02%	-16.34%
10		3	4836	-4669	14340	420	7164	2209	3826	79.12%	40.85%
20		3	8340	8159	8521	8292	8424	42.14	72.99	0.88%	-2.01%
40		3	2920	-1356	7196	972	4236	993.8	1721	58.95%	64.29%
80		3	1260	313.6	2206	972	1692	220	381	30.24%	84.59%

96h Cell Density Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	6	346	306.3	385.7	276	379	15.44	37.83	10.93%	0.0%
5		3	296.7	264.6	328.7	286	311	7.446	12.9	4.35%	14.26%
10		3	207	-236.2	650.2	1	313	103	178.4	86.2%	40.17%
20		3	329.7	299.8	359.5	318	342	6.936	12.01	3.64%	4.72%
40		3	114	-121	349	5	175	54.63	94.62	83.0%	67.05%
80		3	68.33	43.83	92.84	57	75	5.696	9.866	14.44%	80.25%

96h Growth Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	6	0.04423	0.04299	0.04547	0.042	0.0452	0.000483	0.001183	2.67%	0.0%
5		3	0.0427	0.04156	0.04384	0.0423	0.0432	0.000265	0.000458	1.07%	3.47%
10		3	0.02943	-0.0298	0.08867	0.0019	0.0433	0.01377	0.02384	81.01%	33.46%
20		3	0.0438	0.04281	0.04479	0.0434	0.0442	0.000231	0.0004	0.91%	0.98%
40		3	0.027	-0.01561	0.06961	0.0072	0.0373	0.009903	0.01715	63.53%	38.96%
80		3	0.0279	0.02422	0.03158	0.0262	0.0289	0.000854	0.00148	5.3%	36.93%

96h AUC Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	7.416E+3	7.800E+3	8.820E+3	8.016E+3	7.680E+3	9.324E+3
5		1.007E+4	9.696E+3	8.772E+3			
10		4.200E+2	7.164E+3	6.924E+3			
20		8.424E+3	8.304E+3	8.292E+3			
40		4.236E+3	9.720E+2	3.552E+3			
80		9.720E+2	1.692E+3	1.116E+3			

96h Cell Density Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	378	276	379	342	344	357
5		293	286	311			
10		1	307	313			
20		318	342	329			
40		175	5	162			
80		75	73	57			

96h Growth Rate Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	0.0452	0.042	0.0452	0.0442	0.0442	0.0446
5		0.0426	0.0423	0.0432			
10		0.0019	0.0431	0.0433			
20		0.0434	0.0442	0.0438			
40		0.0373	0.0072	0.0365			
80		0.0289	0.0286	0.0262			

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 1 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity							Stillmeadow, Inc.							
Analysis ID: 07-7830-0042	Endpoint: 96h AUC					CETIS Version: CETISv1.8.7								
Analyzed: 11 Feb-14 12:22	Analysis: Nonparametric-Two Sample					Official Results: Yes								
Batch ID: 08-7333-8569	Test Type: Algal Cell Growth (96-h)					Analyst:								
Start Date: 19 Oct-12	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)					Diluent: Algal Culture Media								
Ending Date: 23 Oct-12	Species: Pseudokirchneriella subcapitata					Brine:								
Duration: 96h	Source: Lab In-House Culture					Age: 3-7								
Data Transform	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU				
Untransformed	NA	C > T	NA	NA		26.5%	20	40	28.28					

Mann-Whitney U Two-Sample Test

Control	vs	C-mg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)
Negative Control	5	2	NA	0	7	0.9762	Exact	Non-Significant Effect	
	10*	18	NA	0	7	0.0119	Exact	Significant Effect	
	20	6	NA	0	7	0.8095	Exact	Non-Significant Effect	
	40*	18	NA	0	7	0.0119	Exact	Significant Effect	
	80*	18	NA	0	7	0.0119	Exact	Significant Effect	

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	181127900	36225580	5	13.89	<0.0001	Significant Effect
Error	39121150	2608077	15			
Total	220249000		20			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	21.97	15.09	0.0005	Unequal Variances
Variances	Mod Levene Equality of Variance	1.396	5.636	0.3048	Equal Variances
Variances	Levene Equality of Variance	10.54	4.556	0.0002	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8777	0.871	0.0132	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1982	0.2186	0.0307	Normal Distribution
Distribution	D'Agostino Skewness	2.571	2.576	0.0101	Normal Distribution
Distribution	D'Agostino Kurtosis	2.693	2.576	0.0071	Non-normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	13.86	9.21	0.0010	Non-normal Distribution
Distribution	Anderson-Darling A2 Normality	0.9693	3.878	0.0147	Normal Distribution

96h AUC Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	6	8.176E+3	7.402E+3	8.950E+3	7908	7.416E+3	9.324E+3	3.013E+2	9.03%	0.0%
5		3	9.512E+3	7.854E+3	1.117E+4	9696	8.772E+3	1.007E+4	3.853E+2	7.02%	-16.34%
10		3	4.836E+3	-4.669E+3	1.434E+4	6924	4.200E+2	7.164E+3	2.209E+3	79.12%	40.85%
20		3	8.340E+3	8.159E+3	8.521E+3	8304	8.292E+3	8.424E+3	4.214E+1	0.88%	-2.01%
40		3	2.920E+3	-1.356E+3	7.196E+3	3552	9.720E+2	4.236E+3	9.938E+2	58.95%	64.29%
80		3	1.260E+3	3.136E+2	2.206E+3	1116	9.720E+2	1.692E+3	2.200E+2	30.24%	84.59%

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 2 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

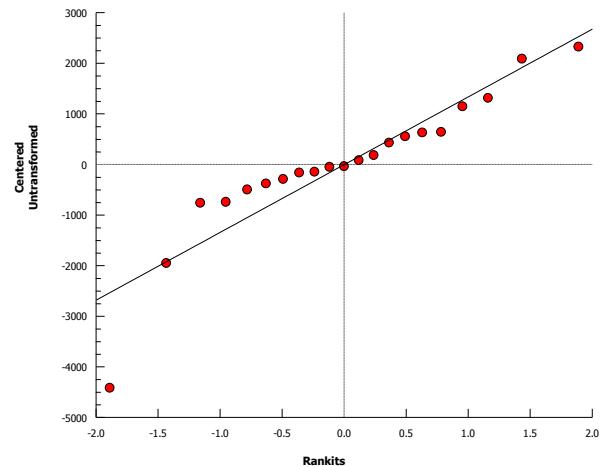
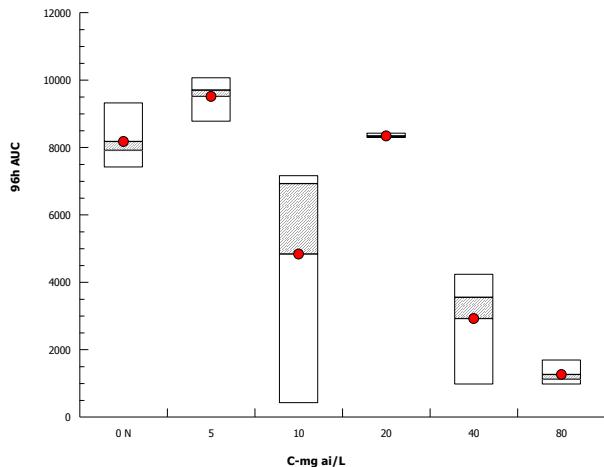
Stillmeadow, Inc.

Analysis ID: 07-7830-0042
Analyzed: 11 Feb-14 12:22

Endpoint: 96h AUC
Analysis: Nonparametric-Two Sample

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 3 of 12)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity								Stillmeadow, Inc.						
Analysis ID:		Endpoint: 96h AUC				CETIS Version:		CETISv1.8.7						
Analyzed:		Analysis: Nonparametric-Control vs Ord. Treatments				Official Results:		Yes						
Batch ID:	08-7333-8569	Test Type: Algal Cell Growth (96-h)				Analyst:								
Start Date:	19 Oct-12	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)				Diluent:		Algal Culture Media						
Ending Date:	23 Oct-12	Species: Pseudokirchneriella subcapitata				Brine:								
Duration:	96h	Source: Lab In-House Culture				Age:		3-7						
Data Transform	Zeta	Alt Hyp	Trials	Seed		NOEL	LOEL	TOEL	TU					
Untransformed	NA	C > T	NA	NA		20	40	28.28						
Jonckheere-Terpstra Step-Down Test														
Control	vs	C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)					
Negative Control	5	2	NA		-2	0.9762	Exact	Non-Significant Effect						
	10	29	NA		-2	0.3799	Exact	Non-Significant Effect						
	20	44	NA		-2	0.3799	Exact	Non-Significant Effect						
	40*	86	NA		-2	0.0391	Exact	Significant Effect						
	80*	2.748	1.645	1	-2	0.0030	Asymp	Significant Effect						
ANOVA Table														
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision(α :5%)					
Between	181127900		36225580		5	13.89		<0.0001	Significant Effect					
Error	39121150		2608077		15									
Total	220249000				20									
Distributional Tests														
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)								
Variances	Bartlett Equality of Variance		21.97	15.09	0.0005	Unequal Variances								
Variances	Mod Levene Equality of Variance		1.396	5.636	0.3048	Equal Variances								
Variances	Levene Equality of Variance		10.54	4.556	0.0002	Unequal Variances								
Distribution	Shapiro-Wilk W Normality		0.8777	0.871	0.0132	Normal Distribution								
Distribution	Kolmogorov-Smirnov D		0.1982	0.2186	0.0307	Normal Distribution								
Distribution	D'Agostino Skewness		2.571	2.576	0.0101	Normal Distribution								
Distribution	D'Agostino Kurtosis		2.693	2.576	0.0071	Non-normal Distribution								
Distribution	D'Agostino-Pearson K2 Omnibus		13.86	9.21	0.0010	Non-normal Distribution								
Distribution	Anderson-Darling A2 Normality		0.9693	3.878	0.0147	Normal Distribution								
96h AUC Summary														
C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect			
0	Negative Control		6	8.176E+3	7.402E+3	8.950E+3	7908	7.416E+3	9.324E+3	3.013E+2	9.03%	0.0%		
5		3	9.512E+3	7.854E+3	1.117E+4	9696	8.772E+3	1.007E+4	3.853E+2	7.02%	-16.34%			
10		3	4.836E+3	-4.669E+3	1.434E+4	6924	4.200E+2	7.164E+3	2.209E+3	79.12%	40.85%			
20		3	8.340E+3	8.159E+3	8.521E+3	8304	8.292E+3	8.424E+3	4.214E+1	0.88%	-2.01%			
40		3	2.920E+3	-1.356E+3	7.196E+3	3552	9.720E+2	4.236E+3	9.938E+2	58.95%	64.29%			
80		3	1.260E+3	3.136E+2	2.206E+3	1116	9.720E+2	1.692E+3	2.200E+2	30.24%	84.59%			

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 4 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

Analysis ID: 17-2946-8890

Endpoint: 96h AUC

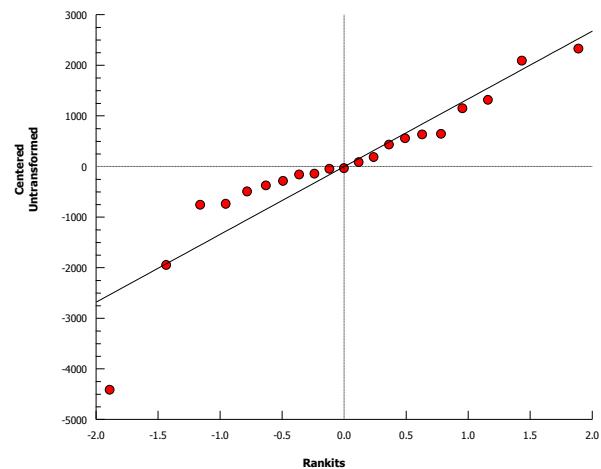
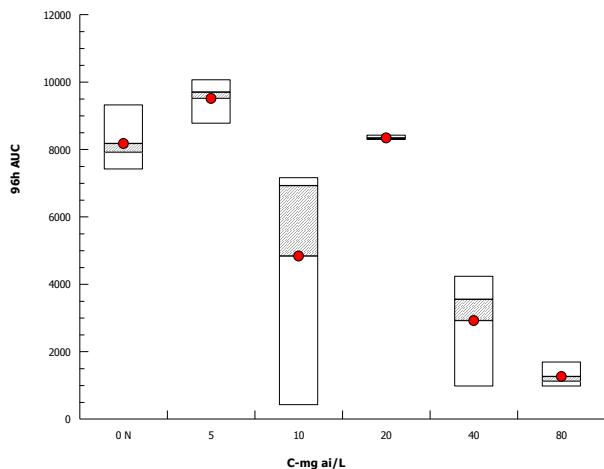
CETIS Version: CETISv1.8.7

Analyzed: 11 Feb-14 12:22

Analysis: Nonparametric-Control vs Ord. Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 5 of 12)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity										Stillmeadow, Inc.								
Analysis ID:		Endpoint: 96h Cell Density				CETIS Version:		CETISv1.8.7										
Analyzed:		Analysis: Nonparametric-Two Sample				Official Results:		Yes										
Batch ID:	08-7333-8569	Test Type: Algal Cell Growth (96-h)				Analyst:												
Start Date:	19 Oct-12	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)				Diluent:		Algal Culture Media										
Ending Date:	23 Oct-12	Species: Pseudokirchneriella subcapitata				Brine:												
Duration:	96h	Source: Lab In-House Culture				Age:		3-7										
Data Transform		Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU								
Untransformed		NA	C > T	NA	NA	29.9%	20	40	28.28									
Mann-Whitney U Two-Sample Test																		
Control	vs	C-mg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)									
Negative Control	5	15	NA	0	7	0.0833	Exact	Non-Significant Effect										
	10*	16	NA	0	7	0.0476	Exact	Significant Effect										
	20	14.5	NA	1	7	0.0952	Exact	Non-Significant Effect										
	40*	18	NA	0	7	0.0119	Exact	Significant Effect										
	80*	18	NA	0	7	0.0119	Exact	Significant Effect										
ANOVA Table																		
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α :5%)										
Between	240145		48028.99		5	8.045	0.0007	Significant Effect										
Error	89548		5969.867		15													
Total	329692.9				20													
Distributional Tests																		
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)												
Variances	Bartlett Equality of Variance		21.97	15.09	0.0005	Unequal Variances												
Variances	Mod Levene Equality of Variance		1.256	5.636	0.3538	Equal Variances												
Variances	Levene Equality of Variance		9.947	4.556	0.0002	Unequal Variances												
Distribution	Shapiro-Wilk W Normality		0.8512	0.871	0.0045	Non-normal Distribution												
Distribution	Kolmogorov-Smirnov D		0.2879	0.2186	<0.0001	Non-normal Distribution												
Distribution	D'Agostino Skewness		2.654	2.576	0.0080	Non-normal Distribution												
Distribution	D'Agostino Kurtosis		2.547	2.576	0.0109	Normal Distribution												
Distribution	D'Agostino-Pearson K2 Omnibus		13.53	9.21	0.0012	Non-normal Distribution												
Distribution	Anderson-Darling A2 Normality		1.418	3.878	0.0006	Non-normal Distribution												
96h Cell Density Summary																		
C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect							
0	Negative Control	6	346	306.3	385.7	350.5	276	379	15.44	10.93%	0.0%							
5		3	296.7	264.6	328.7	293	286	311	7.446	4.35%	14.26%							
10		3	207	-236.2	650.2	307	1	313	103	86.2%	40.17%							
20		3	329.7	299.8	359.5	329	318	342	6.936	3.64%	4.72%							
40		3	114	-121	349	162	5	175	54.63	83.0%	67.05%							
80		3	68.33	43.83	92.84	73	57	75	5.696	14.44%	80.25%							

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 6 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

Analysis ID: 01-3284-6640

Endpoint: 96h Cell Density

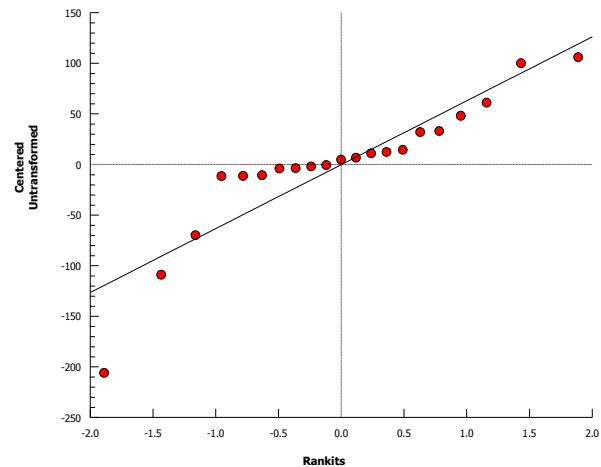
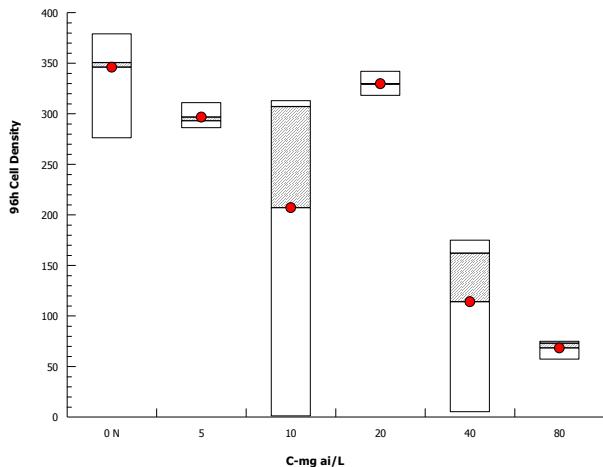
CETIS Version: CETISv1.8.7

Analyzed: 11 Feb-14 12:21

Analysis: Nonparametric-Two Sample

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 7 of 12)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity							Stillmeadow, Inc.			
Analysis ID:		Endpoint:			CETIS Version:		CETISv1.8.7			
Analyzed:		Analysis:			Official Results:		Yes			
Batch ID:	08-7333-8569	Test Type:			Analyst:					
Start Date:	19 Oct-12	Protocol:			Diluent:			Algal Culture Media		
Ending Date:	23 Oct-12	Species:			Brine:					
Duration:	96h	Source:			Age:			3-7		
Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU		
Untransformed	NA	C > T	NA	NA	20	40	28.28			

Jonckheere-Terpstra Step-Down Test

Control	vs	C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Negative Control	5	15	NA		-2	0.1740	Exact	Non-Significant Effect	
	10	35	NA		-2	0.1740	Exact	Non-Significant Effect	
	20	0.9383	1.645	1	-2	0.1740	Asymp	Non-Significant Effect	
	40*	2.231	1.645	1	-2	0.0129	Asymp	Significant Effect	
	80*	3.057	1.645	1	-2	0.0011	Asymp	Significant Effect	

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	240145	48028.99	5	8.045	0.0007	Significant Effect
Error	89548	5969.867	15			
Total	329692.9		20			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	21.97	15.09	0.0005	Unequal Variances
Variances	Mod Levene Equality of Variance	1.256	5.636	0.3538	Equal Variances
Variances	Levene Equality of Variance	9.947	4.556	0.0002	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8512	0.871	0.0045	Non-normal Distribution
Distribution	Kolmogorov-Smirnov D	0.2879	0.2186	<0.0001	Non-normal Distribution
Distribution	D'Agostino Skewness	2.654	2.576	0.0080	Non-normal Distribution
Distribution	D'Agostino Kurtosis	2.547	2.576	0.0109	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	13.53	9.21	0.0012	Non-normal Distribution
Distribution	Anderson-Darling A2 Normality	1.418	3.878	0.0006	Non-normal Distribution

96h Cell Density Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	6	346	306.3	385.7	350.5	276	379	15.44	10.93%	0.0%
5		3	296.7	264.6	328.7	293	286	311	7.446	4.35%	14.26%
10		3	207	-236.2	650.2	307	1	313	103	86.2%	40.17%
20		3	329.7	299.8	359.5	329	318	342	6.936	3.64%	4.72%
40		3	114	-121	349	162	5	175	54.63	83.0%	67.05%
80		3	68.33	43.83	92.84	73	57	75	5.696	14.44%	80.25%

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 8 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

Analysis ID: 18-6441-3558

Endpoint: 96h Cell Density

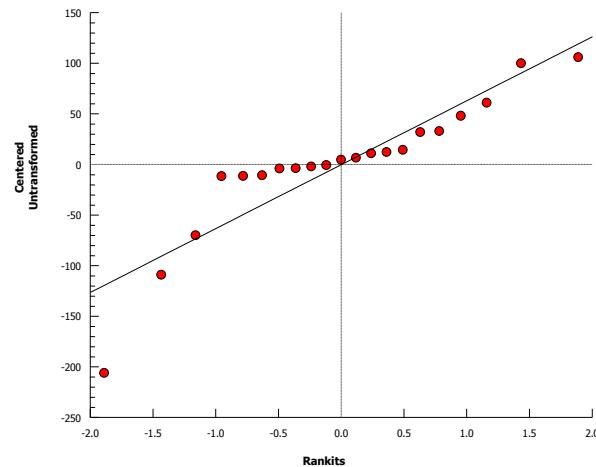
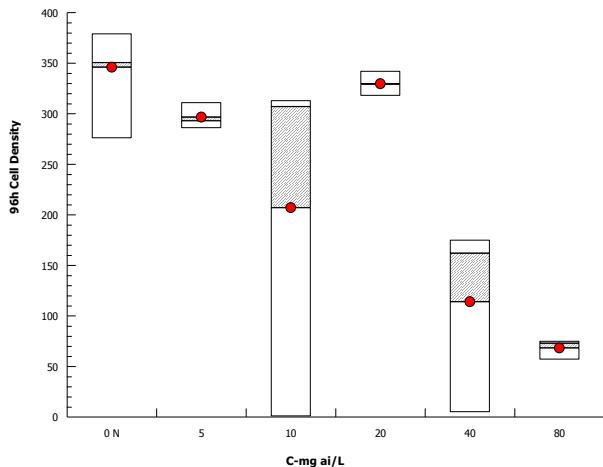
CETIS Version: CETISv1.8.7

Analyzed: 11 Feb-14 12:22

Analysis: Nonparametric-Control vs Ord. Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 9 of 12)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity										Stillmeadow, Inc.						
Analysis ID:		19-0981-3494	Endpoint: 96h Growth Rate				CETIS Version:		CETISv1.8.7							
Analyzed:		11 Feb-14 12:21	Analysis: Nonparametric-Two Sample				Official Results:		Yes							
Batch ID:	08-7333-8569	Test Type:	Algal Cell Growth (96-h)				Analyst:									
Start Date:	19 Oct-12	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)				Diluent:	Algal Culture Media								
Ending Date:	23 Oct-12	Species:	Pseudokirchneriella subcapitata				Brine:									
Duration:	96h	Source:	Lab In-House Culture				Age:	3-7								
Data Transform	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU						
Untransformed	NA	C > T	NA	NA		32.6%	20	40	28.28							
Mann-Whitney U Two-Sample Test																
Control	vs	C-mg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision($\alpha:5\%$)							
Negative Control	5	15	NA	0	7	0.0952	Exact	Non-Significant Effect								
	10*	16	NA	0	7	0.0357	Exact	Significant Effect								
	20	14	NA	1	7	0.1310	Exact	Non-Significant Effect								
	40*	18	NA	0	7	0.0119	Exact	Significant Effect								
	80*	18	NA	0	7	0.0119	Exact	Significant Effect								
ANOVA Table																
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision($\alpha:5\%$)							
Between	0.001270271		0.0002540543		5	2.193		0.1096	Non-Significant Effect							
Error	0.00173764		0.0001158427		15											
Total	0.003007911				20											
Distributional Tests																
Attribute	Test		Test Stat	Critical	P-Value	Decision($\alpha:1\%$)										
Variances	Bartlett Equality of Variance		43.19	15.09	<0.0001	Unequal Variances										
Variances	Mod Levene Equality of Variance		1.458	5.636	0.2857	Equal Variances										
Variances	Levene Equality of Variance		15.62	4.556	<0.0001	Unequal Variances										
Distribution	Shapiro-Wilk W Normality		0.7546	0.871	0.0001	Non-normal Distribution										
Distribution	Kolmogorov-Smirnov D		0.3101	0.2186	<0.0001	Non-normal Distribution										
Distribution	D'Agostino Skewness		2.698	2.576	0.0070	Non-normal Distribution										
Distribution	D'Agostino Kurtosis		2.506	2.576	0.0122	Normal Distribution										
Distribution	D'Agostino-Pearson K2 Omnibus		13.56	9.21	0.0011	Non-normal Distribution										
Distribution	Anderson-Darling A2 Normality		2.675	3.878	<0.0001	Non-normal Distribution										
96h Growth Rate Summary																
C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect					
0	Negative Control		6	0.04423	0.04299	0.04547	0.0444	0.042	0.0452	0.000483	2.67%					
5		3	0.0427	0.04156	0.04384	0.0426	0.0423	0.0432	0.000265	1.07%	3.47%					
10		3	0.02943	-0.0298	0.08867	0.0431	0.0019	0.0433	0.01377	81.01%	33.46%					
20		3	0.0438	0.04281	0.04479	0.0438	0.0434	0.0442	0.000231	0.91%	0.98%					
40		3	0.027	-0.01561	0.06961	0.0365	0.0072	0.0373	0.009903	63.53%	38.96%					
80		3	0.0279	0.02422	0.03158	0.0286	0.0262	0.0289	0.000854	5.3%	36.93%					

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 10 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

Analysis ID: 19-0981-3494

Endpoint: 96h Growth Rate

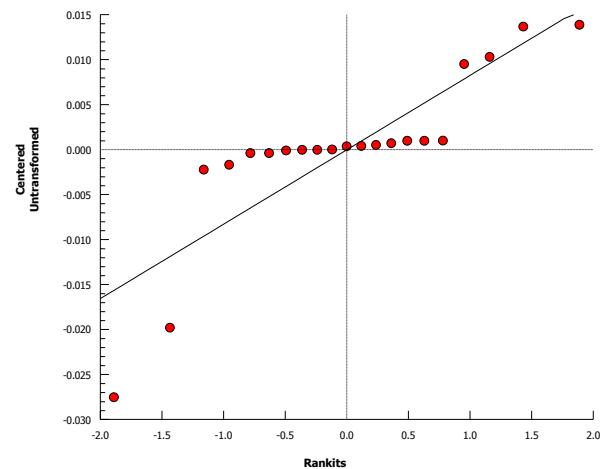
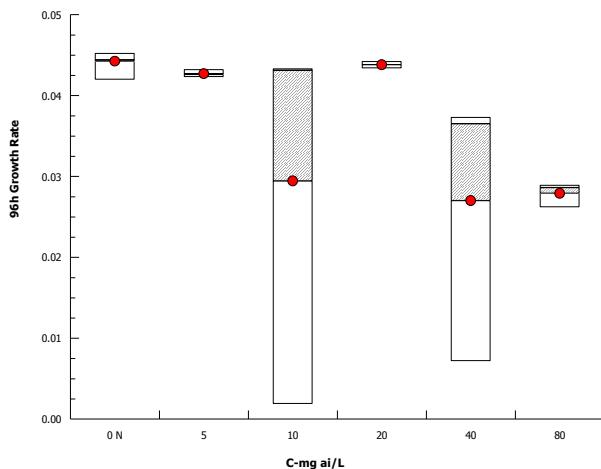
CETIS Version: CETISv1.8.7

Analyzed: 11 Feb-14 12:21

Analysis: Nonparametric-Two Sample

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 11 of 12)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity										Stillmeadow, Inc.						
Analysis ID:		Endpoint: 96h Growth Rate				CETIS Version:		CETISv1.8.7								
Analyzed:		Analysis: Nonparametric-Control vs Ord. Treatments				Official Results:		Yes								
Batch ID:	08-7333-8569	Test Type: Algal Cell Growth (96-h)				Analyst:										
Start Date:	19 Oct-12	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)				Diluent:		Algal Culture Media								
Ending Date:	23 Oct-12	Species: Pseudokirchneriella subcapitata				Brine:										
Duration:	96h	Source: Lab In-House Culture				Age:		3-7								
Data Transform		Zeta	Alt Hyp	Trials	Seed	NOEL		LOEL	TOEL	TU						
Untransformed		NA	C > T	NA	NA	20		40	28.28							
Jonckheere-Terpstra Step-Down Test																
Control	vs	C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)							
Negative Control	5	15	NA		-2	0.1869	Exact	Non-Significant Effect								
	10	35	NA		-2	0.1869	Exact	Non-Significant Effect								
	20	0.8894	1.645	1	-2	0.1869	Asymp	Non-Significant Effect								
	40*	2.196	1.645	1	-2	0.0140	Asymp	Significant Effect								
	80*	3.03	1.645	1	-2	0.0012	Asymp	Significant Effect								
ANOVA Table																
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision(α :5%)							
Between	0.001270271		0.0002540543		5	2.193		0.1096	Non-Significant Effect							
Error	0.00173764		0.0001158427		15											
Total	0.003007911				20											
Distributional Tests																
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)										
Variances	Bartlett Equality of Variance		43.19	15.09	<0.0001	Unequal Variances										
Variances	Mod Levene Equality of Variance		1.458	5.636	0.2857	Equal Variances										
Variances	Levene Equality of Variance		15.62	4.556	<0.0001	Unequal Variances										
Distribution	Shapiro-Wilk W Normality		0.7546	0.871	0.0001	Non-normal Distribution										
Distribution	Kolmogorov-Smirnov D		0.3101	0.2186	<0.0001	Non-normal Distribution										
Distribution	D'Agostino Skewness		2.698	2.576	0.0070	Non-normal Distribution										
Distribution	D'Agostino Kurtosis		2.506	2.576	0.0122	Normal Distribution										
Distribution	D'Agostino-Pearson K2 Omnibus		13.56	9.21	0.0011	Non-normal Distribution										
Distribution	Anderson-Darling A2 Normality		2.675	3.878	<0.0001	Non-normal Distribution										
96h Growth Rate Summary																
C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect					
0	Negative Control	6	0.04423	0.04299	0.04547	0.0444	0.042	0.0452	0.000483	2.67%	0.0%					
5		3	0.0427	0.04156	0.04384	0.0426	0.0423	0.0432	0.000265	1.07%	3.47%					
10		3	0.02943	-0.0298	0.08867	0.0431	0.0019	0.0433	0.01377	81.01%	33.46%					
20		3	0.0438	0.04281	0.04479	0.0438	0.0434	0.0442	0.000231	0.91%	0.98%					
40		3	0.027	-0.01561	0.06961	0.0365	0.0072	0.0373	0.009903	63.53%	38.96%					
80		3	0.0279	0.02422	0.03158	0.0286	0.0262	0.0289	0.000854	5.3%	36.93%					

CETIS Analytical Report

Report Date: 11 Feb-14 12:24 (p 12 of 12)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

Analysis ID: 04-1742-7163

Endpoint: 96h Growth Rate

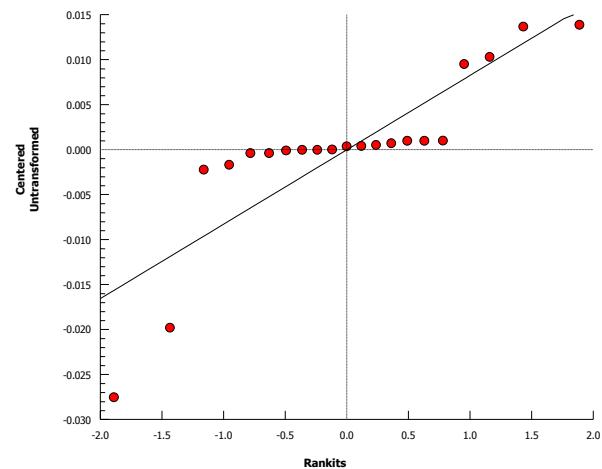
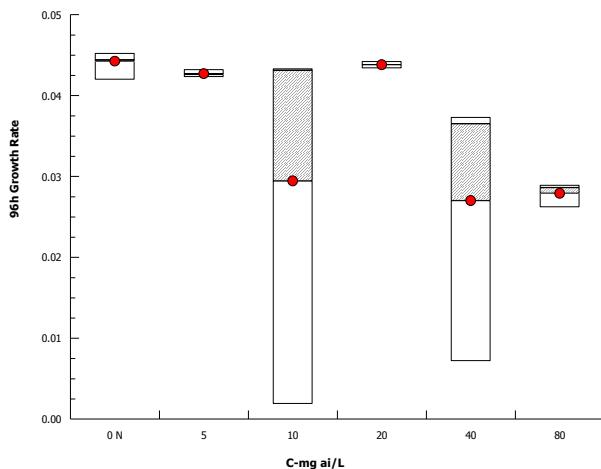
CETIS Version: CETISv1.8.7

Analyzed: 11 Feb-14 12:22

Analysis: Nonparametric-Control vs Ord. Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Feb-14 12:28 (p 1 of 6)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity**Stillmeadow, Inc.**

Analysis ID: 04-7375-0817 **Endpoint:** 96h AUC **CETIS Version:** CETISv1.8.7
Analyzed: 11 Feb-14 12:22 **Analysis:** Nonlinear Regression **Official Results:** Yes

Batch ID: 08-7333-8569 **Test Type:** Algal Cell Growth (96-h) **Analyst:**
Start Date: 19 Oct-12 **Protocol:** OCSPP 850.4500 Aquatic Plant (Algae) **Diluent:** Algal Culture Media
Ending Date: 23 Oct-12 **Species:** Pseudokirchneriella subcapitata **Brine:**
Duration: 96h **Source:** Lab In-House Culture **Age:** 3-7

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Poisson [W=1/Y]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
11	-9238	18480	18480	0.6146	Yes	2.076	3.287	0.1465	Non-Significant Lack of Fit

Point Estimates

Level	mg ai/L	95% LCL	95% UCL
IC5	11.28	N/A	20.25
IC10	14.64	N/A	24.17
IC25	22.64	9.416	34.88
IC50	36.75	26.48	50.99

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	8069	732.9	6633	9506	11.01	<0.0001	Significant Parameter
C	0.7182	0.2174	0.2921	1.144	3.304	0.0040	Significant Parameter
D	36.75	7.211	22.61	50.88	5.096	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	23300.28	23300.28	1	33.9	<0.0001	Significant
Lack of Fit	3629.21	1209.737	3	2.076	0.1465	Non-Significant
Pure Error	8742.499	582.8333	15			
Residual	12371.71	687.3172	18			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	12370	28.87	<0.0001	Significant Heterogeneity
	Likelihood Ratio GOF	18260	28.87	<0.0001	Significant Heterogeneity
Variances	Mod Levene Equality of Variance	1.416	3.326	0.2987	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7677	0.9079	0.0002	Non-normal Distribution
	Anderson-Darling A2 Normality	1.665	2.492	<0.0001	Non-normal Distribution

96h AUC Summary

C-mg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	6	8.176E+3	7.416E+3	9.324E+3	3.013E+2	7.380E+2	9.03%	0.0%
5		3	9.512E+3	8.772E+3	1.007E+4	3.853E+2	6.673E+2	7.02%	-16.34%
10		3	4.836E+3	4.200E+2	7.164E+3	2.209E+3	3.826E+3	79.12%	40.85%
20		3	8.340E+3	8.292E+3	8.424E+3	4.214E+1	7.299E+1	0.88%	-2.01%
40		3	2.920E+3	9.720E+2	4.236E+3	9.938E+2	1.721E+3	58.95%	64.29%
80		3	1.260E+3	9.720E+2	1.692E+3	2.200E+2	3.810E+2	30.24%	84.59%

CETIS Analytical Report

Report Date: 11 Feb-14 12:28 (p 2 of 6)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

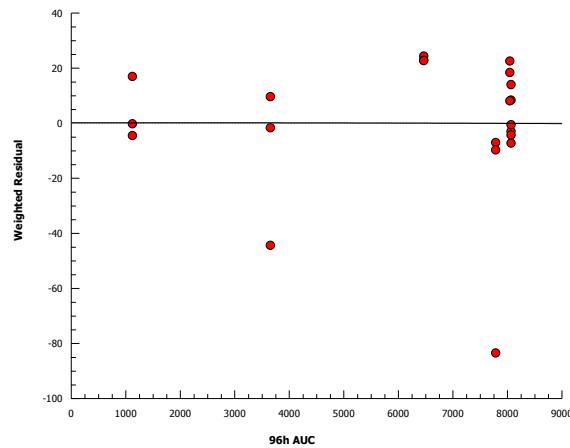
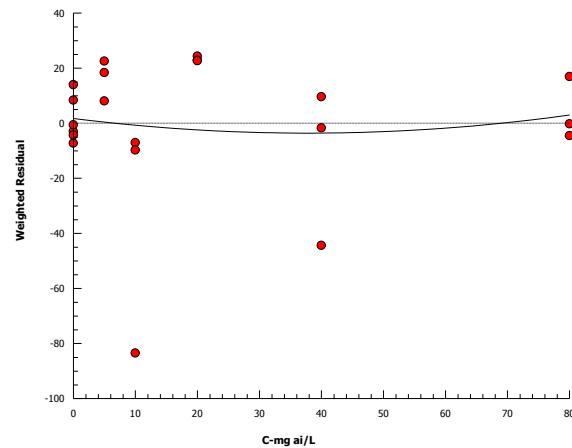
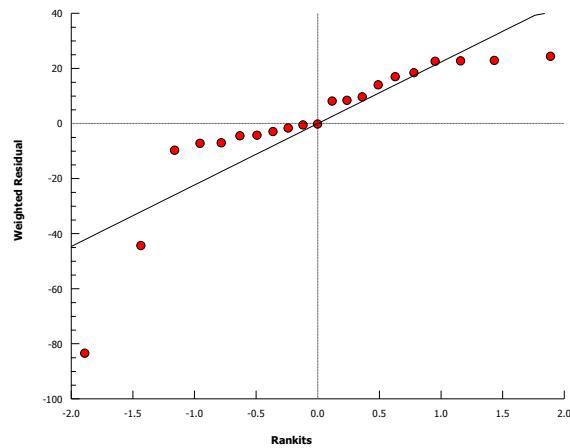
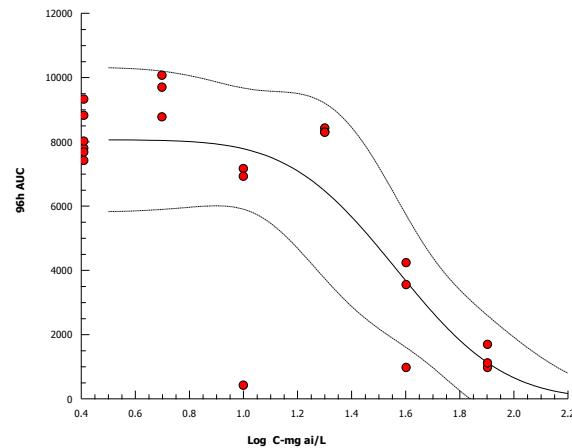
Analysis ID: 04-7375-0817
Analyzed: 11 Feb-14 12:22

Endpoint: 96h AUC
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Analytical Report

Report Date: 11 Feb-14 12:28 (p 3 of 6)
Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity										Stillmeadow, Inc.					
Analysis ID:	12-9972-1746	Endpoint:	96h Cell Density					CETIS Version:	CETISv1.8.7						
Analyzed:	11 Feb-14 12:21	Analysis:	Nonlinear Regression					Official Results:	Yes						
Batch ID:	08-7333-8569	Test Type:	Algal Cell Growth (96-h)					Analyst:							
Start Date:	19 Oct-12	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)					Diluent:	Algal Culture Media						
Ending Date:	23 Oct-12	Species:	Pseudokirchneriella subcapitata					Brine:							
Duration:	96h	Source:	Lab In-House Culture					Age:	3-7						
Non-Linear Regression Options															
Model Function				X Transform	Y Transform	Weighting Function	PTBS Function								
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]				None	None	Poisson [W=1/Y]	Off [Y*=Y]								
Regression Summary															
Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)						
18	-558.3	1124	1126	0.5216	Yes	0.9351	3.287	0.4481	Non-Significant Lack of Fit						
Point Estimates															
Level	mg ai/L	95% LCL	95% UCL												
IC5	8.631	N/A	18.54												
IC10	11.93	N/A	22.77												
IC25	20.47	6.377	35.4												
IC50	37.31	25.04	55.59												
Regression Parameters															
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)								
A	319.7	34.04	253	386.4	9.391	<0.0001	Significant Parameter								
C	0.89	0.3215	0.2599	1.52	2.768	0.0127	Significant Parameter								
D	37.31	9.447	18.79	55.83	3.949	0.0009	Significant Parameter								
ANOVA Table															
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)									
Model	769.6019	769.6019	1	23.81	0.0001	Significant									
Lack of Fit	91.67602	30.55867	3	0.9351	0.4481	Non-Significant									
Pure Error	490.1864	32.6791	15												
Residual	581.8625	32.32569	18												
Residual Analysis															
Attribute	Method		Test Stat	Critical	P-Value	Decision(α:5%)									
Goodness-of-Fit	Pearson Chi-Sq GOF		581.9	28.87	<0.0001	Significant Heterogeneity									
	Likelihood Ratio GOF		972.2	28.87	<0.0001	Significant Heterogeneity									
Variances	Mod Levene Equality of Variance	1.256	3.326	0.3537	Equal Variances										
	Shapiro-Wilk W Normality	0.7591	0.9079	0.0002	Non-normal Distribution										
Distribution	Anderson-Darling A2 Normality	2.086	2.492	<0.0001	Non-normal Distribution										
96h Cell Density Summary															
Calculated Variate															
C-mg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect						
0	Negative Control	6	346	276	379	15.44	37.83	10.93%	0.0%						
5		3	296.7	286	311	7.446	12.9	4.35%	14.26%						
10		3	207	1	313	103	178.4	86.2%	40.17%						
20		3	329.7	318	342	6.936	12.01	3.64%	4.72%						
40		3	114	5	175	54.63	94.62	83.0%	67.05%						
80		3	68.33	57	75	5.696	9.866	14.44%	80.25%						

CETIS Analytical Report

Report Date: 11 Feb-14 12:28 (p 4 of 6)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

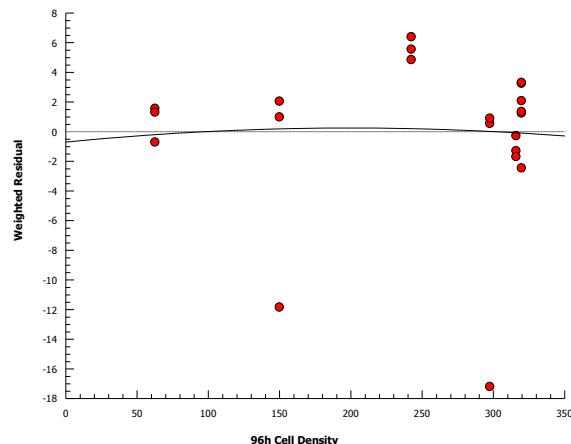
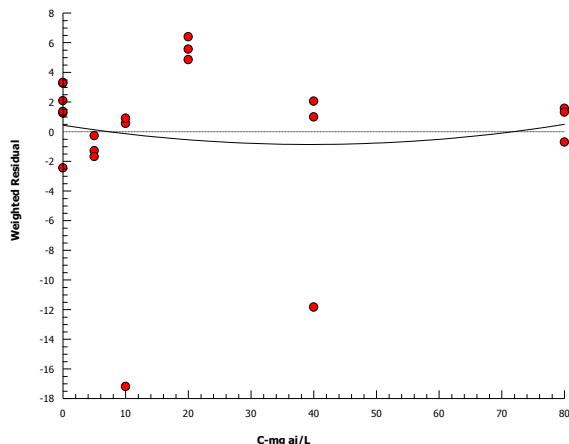
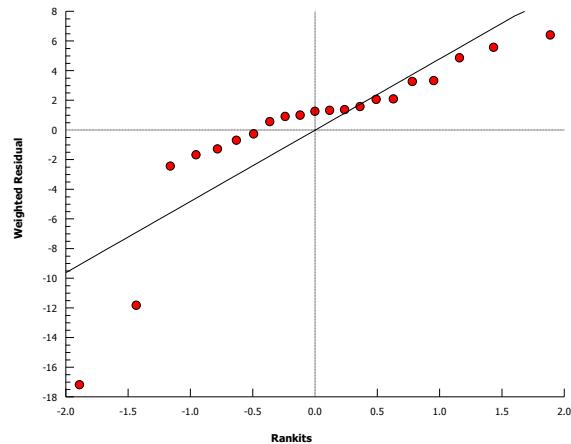
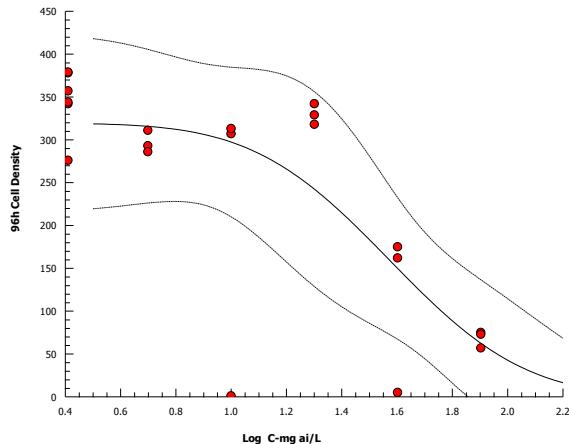
Analysis ID: 12-9972-1746
 Analyzed: 11 Feb-14 12:21

Endpoint: 96h Cell Density
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Analytical Report

Report Date: 11 Feb-14 12:28 (p 5 of 6)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity										Stillmeadow, Inc.									
Analysis ID: 08-1788-5182 Analyzed: 11 Feb-14 12:21			Endpoint: 96h Growth Rate Analysis: Nonlinear Regression				CETIS Version: CETISv1.8.7 Official Results: Yes												
Batch ID: 08-7333-8569	Test Type: Algal Cell Growth (96-h)				Analyst:			Diluent: Algal Culture Media											
Start Date: 19 Oct-12	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)				Species: Pseudokirchneriella subcapitata			Brine:											
Ending Date: 23 Oct-12	Source: Lab In-House Culture				Age: 3-7														
Non-Linear Regression Options																			
Model Function				X Transform		Y Transform	Weighting Function		PTBS Function										
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]				None		None	Poisson [W=1/Y]		Off [Y*=Y]										
Regression Summary																			
Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α :5%)										
4	-3.331	14.07	15.8	0.1224	Yes	0.278	3.287	0.8404	Non-Significant Lack of Fit										
Point Estimates																			
Level	mg ai/L	95% LCL	95% UCL																
IC5	1.917	N/A	22.71																
IC10	5.22	N/A	37.16																
IC25	27.83	6.243	81.62																
IC50	178.7	8.885	3593																
Regression Parameters																			
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat		P-Value	Decision(α :5%)											
A	0.04423	0.005079	0.03428	0.05418	8.709	<0.0001	Significant	Parameter											
C	2.757	2.146	-1.45	6.964	1.284	0.2153	Non-Significant	Parameter											
D	178.7	226.9	-266.1	623.5	0.7873	0.4413	Non-Significant	Parameter											
ANOVA Table																			
Source	Sum Squares	Mean Square	DF	F Stat		P-Value	Decision(α :5%)												
Model	0.017064	0.017064	1	4.789		0.0421	Significant												
Lack of Fit	0.003378	0.001126	3	0.278		0.8404	Non-Significant												
Pure Error	0.060759	0.004051	15																
Residual	0.064137	0.003563	18																
Residual Analysis																			
Attribute	Method		Test Stat		Critical	P-Value	Decision(α :5%)												
Goodness-of-Fit	Pearson Chi-Sq GOF		0.06414		28.87	1.0000	Non-Significant Heterogeneity												
	Likelihood Ratio GOF		0.09809		28.87	1.0000	Non-Significant Heterogeneity												
Variances	Mod Levene Equality of Variance		1.46		3.326	0.2851	Equal Variances												
	Distribution		Shapiro-Wilk W Normality		0.6548	0.9079	<0.0001												
	Anderson-Darling A2 Normality		3.119		2.492	<0.0001	Non-normal Distribution												
96h Growth Rate Summary																			
			Calculated Variate																
C-mg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect										
0	Negative Control		0.04423	0.042	0.0452	0.000483	0.001183	2.67%	0.0%										
5			0.0427	0.0423	0.0432	0.000265	0.000458	1.07%	3.47%										
10			0.02943	0.0019	0.0433	0.01377	0.02384	81.01%	33.46%										
20			0.0438	0.0434	0.0442	0.000231	0.0004	0.91%	0.98%										
40			0.027	0.0072	0.0373	0.009903	0.01715	63.53%	38.96%										
80			0.0279	0.0262	0.0289	0.000854	0.00148	5.3%	36.93%										

CETIS Analytical Report

Report Date: 11 Feb-14 12:28 (p 6 of 6)
 Test Code: 079701 49044002 | 21-2198-6698

OCSPP 850.4500 Algal Toxicity

Stillmeadow, Inc.

Analysis ID: 08-1788-5182
 Analyzed: 11 Feb-14 12:21

Endpoint: 96h Growth Rate
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]

